Prediction of Spinach Quality Based on Pre- and Postharvest Conditions

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Keywords: spinach, nitrogen, organic acids, ammonium, glutamine synthetase, storage

Abstract

Nitrogen amendments and water availability have a significant effect on the nutritional quality of spinach. Despite this established knowledge, the specific influence of these interacting factors on overall postharvest quality is poorly understood. Determined by interviews with multiple growers, current agronomic practices in California and Arizona routinely include the application of 100 to 350 kg/ha total N. Preharvest trials and postharvest evaluations with field and hydroponically-grown spinach were conducted to elucidate how this fertility management practice influences quality. Additionally, quantitative evaluations were performed to identify predictors of market quality at harvest and during storage. Preharvest nitrogen availability greater than 100 ppm coupled with high temperature during cultivation reduced the overall quality of spinach. Color (hue) of spinach grown under various N rates and stored at 7.5°C (in perforated polymer-film packaging) did not reliably predict the key phytonutrient composition and marketable shelf life. Marketable shelf life was initially defined as a combination of visual quality score (OVQ) ≥6.5 (on a 9 point hedonic scale), decay <1%, and ascorbic acid concentrations ≥35 mg/100 g FW. Ammonium accumulation more reliably anticipated the reduction of quality during storage than other parameters taken as single assessment indicators. Changes in respiration rates, chlorophyll and carotenoid concentrations during storage were poor indicators of marketable shelf life. More accurate quality retention projections were achieved by combining ammonium concentrations with measurements of glutamine synthetase (GS) activity at harvest. Total organic acids (TOA) and sugar content >3.5 mg/g FW at harvest were negatively associated with quality retention. Although storage temperatures significantly affect quality retention in spinach, the initial quality at harvest is the main factor that influences inherent postharvest potential. Spinach pre- and postharvest quality is best predicted by analysis of TOA, sugar concentrations and the relationship between ammonium and GS at harvest while during storage OVQ, TOA and ammonium concentrations were practical descriptors of marketable shelf life.

INTRODUCTION

Postharvest management for spinach focuses mainly on temperature control and water loss. Because of its high respiration rates (40-70 ml CO_2/kg-h at 10°C) and its high sensitivity to ethylene and elevated CO_2 concentrations, spinach needs to be cooled in an expedited manner soon after harvest (Suslow and Cantwell, 2009). Once spinach has been packaged, high oxygen and low carbon dioxide concentrations at temperatures close to 0°C favor high spinach quality (Cantwell et al., 1998; Suslow and Cantwell, 2009). There have been several attempts to describe the postharvest quality of spinach during storage at different temperatures based on color and visual quality measurements as well as chlorophyll, and carotenoid content. Although reproducible results were described, the marked differences in storage temperatures were the driving factor in the quality assessments reported. The objective of this study was to characterize those quality assessments at harvest that could predict the postharvest quality of spinach during storage associated with N fertilization.

MATERIALS AND METHODS

Greenhouse and field spinach cultivation was conducted at the University of California Davis and in commercial fields within the Salinas Valley, California respectively. Under greenhouse conditions; spinach seeds (*Spinacia oleracea* L. 'Whale') were sown into a 50:50 coir-vermiculite medium with a moisture content of 3:1 (soil-water ratio). The photoperiod varied between 13 and 15 h a day according to growing season. The temperature ranged between 15-28°C night/day and average relative humidity was 65%. The growing period was 30-35 days during the months of May and August 2006. Plants were transplanted into the hydroponic system when they had 6 fully expanded leaves and each plant was placed in a 5-cm wide coir cone for support. Each hydroponic system consisted of 7.5-L buckets with a white lid holding 4 plants per pot with 7.5 cm between plants. Treatments consisted of 2 nutrient solutions, each with different nitrogen content (125 and 200 ppm total N) but with identical nitrate to ammonium ratio (80:20). The pH and Ec were monitored and pH adjusted every two days to the target pH of 5.8. Once per week the nutrient solution was replaced with new solution. Field growing conditions of spinach ('Avenger') followed normal commercial practices during the months of June and July 2008 in the Salinas Valley with temperature fluctuation between 20 and 40°C. Total nitrogen fertilization regimes were 190 and 270 kg/ha. Plants were harvested at dawn by hand, in parallel with a commercial harvest.

Greenhouse or field cultivated spinach at harvest was divided into subsamples and stored at 7.5°C in perforated polyethylene bags. Postharvest evaluations were done at 3 to 7 day intervals for a period of up to 25 days. Chlorophyll and carotenoid determination was done from finely chopped spinach (3 g) placed in a 50 ml falcon tube following the procedure described by (Lichtenthaler, 1987). Total sugars from 3 g of spinach were extracted with 17 ml of 95% methanol following the method of Buysse et al. (1993).

Ammonium in leaf tissue was determined on 3 g of fresh tissue placed into a Falcon™ tube and stored at -80°C until analysis following the procedure described Beecher and Whitten (1970). Glutamine synthetase (GS) activity was determined by a method modified from Downs et al. (1994). Ascorbic acid was determined on cold 2% oxalic acid extracts, filtered and frozen at -80°C for subsequent analysis by HPLC. TOA were extracted from five grams of dry weight tissue with 16 ml of nanopure water following a modified method from Gökmen et al. (2000) and measured by HPLC. Respiration was measured from 65 to 75 g of spinach placed in plastic containers connected to a humidified air (~95% RH) stream at 7.5°C, controlled by capillaries at flow rates calculated to maintain CO_2 concentrations <0.5%. The CO_2 concentrations were monitored by taking 1 ml gas samples and injecting into an infrared gas analyzer (Horiba PIR-2000, Horiba, Irvine, CA). For calibration, a standard mixture of 0.547% CO_2 was used. Visual quality was scored as described by Cantwell et al. (1998). Decay was estimated as percentage of the total weight of the tissue inside the bag. Color changes were monitored with a Minolta color meter using L*, a* and b* color values. Hue angles (H) were calculated as $\tan^{-1}$ (b*/a*); 2), from measurements made on the top right side of leaves and correlated with chlorophyll content.

Leaf number 8 and 16 for greenhouse trials and leaf number 1-2 and 3-4 for field trials were used in the anatomical evaluations. From the right side of the adaxial leaf surface a 1×1 cm piece was excised. Each piece was held in a slit-support block made from a carrot root, and leaf sections of 150 μm were obtained with a microtome (Vibratome Series 1000 plus). Each section was photographed using an Olympus U-PMTVC BH-2 microscope at 4 and 10 objective magnification. Total leaf and epidermis thickness were measured from 8 independent replicates. Cuticle was stained using Nile Red dye (5×10^3 mg/ml) solution. Five leaf cross sections were stained and photographed at 100× objective magnification.

RESULTS AND DISCUSSION

Spinach grown in hydroponic culture with 125 and 200 ppm total N had similar nutritional composition as spinach grown in the Salinas Valley under comparable levels of

nitrogen fertilization. Total sugars, TOA and ammonium concentrations were good indicators of the overall nutritional quality and shelf life of spinach at harvest and during storage. Sugar concentrations below 3 mg/g FW as well as TOA equal to or higher than 35 mg/g DW at harvest (Table 1) were associated with reduced nutritional quality. Regression analysis of the main quality characteristics (Table 4) indicated that the relationship between OVQ and decay, ammonium and vitamin C could be used to predict shelf life. However, the relationship of Hue-chlorophyll (y=0.049x-4.744, r=0.2087 and a probability of 0.69) and OVQ-chlorophyll (Table 4) were poor indicators of quality at harvest and during storage. Temperatures during cultivation were 10°C higher under field conditions than under hydroponic conditions. Gruda (2005) reported that increase in temperature during cultivation drastically alters plant development and negatively impacts crop quality.

Increasing nitrogen fertilization in hydroponic culture or field conditions by 30% did not increase chlorophyll and carotenoid concentrations significantly among treatments. Carotenoid levels, presumably key phytonutrients, remained stable during storage; and as with chlorophyll were not observed to be good indicators of postharvest shelf life (Figs. 2 and 3). OVQ scores were similar between N treatments within the first 7 days of storage with all cultivation methods, but significantly different after 14 days. Hydroponic spinach after 20 days of storage had greater visual quality scores than field experimental production or commercial spinach. Decay scores after 10 days for all N treatments, cultivation methods and spinach sources were greater than 1% (Figs. 2 and 3). Cantwell et al. (1998) reported that decay scores greater than 1% in spinach would render unitized packaged forms of this leafy green unmarketable to consumers. There was a discrepancy between the OVQ and decay scores from hydroponic spinach and to a lesser extent with field plot samples. At 10 days or later OVQ scores still indicated that spinach was marketable while decay scores indicated the opposite. Nonetheless, there was strong relationship between these two parameters and spinach shelf life (Table 4). This discrepancy could be partially explained by differences in the level of processing when compared to retail samples (Cantwell et al., 1998).

High nitrogen supply resulted in greater ammonium content and greater GS activity in spinach at harvest due to additional ammonium availability during cultivation. Greater ammonium concentrations and GS activity at harvest were associated with reduced postharvest shelf life (Table 4, Figs. 1 and 2). The estimated GS activity was consistent with values reported by Cruz et al. (2006). Although this relationship was not estimated for field grown plants, due to greater ammonium availability during cultivation these plants also had higher ammonium concentrations at harvest and during storage when compared to hydroponic spinach (Figs. 2 and 3). Vitamin C degradation during storage was significant in all N treatments. However, concentrations after 21 days of storage did not reach levels below those recommended (35 mg/100 g FW) by the USDA (2010). Changes in green color of spinach during storage were marginal between nitrogen treatments and were poor indicators of spinach postharvest shelf life (Table 4). Hue values remained constant (Hue=125.4±1.0) during 15 days of storage for both N treatments. Spinach processed and packed in the Salinas Valley for retail or foodservice markets followed a similar trend in green color measurements (Hue) during storage as that for hydroponic spinach, despite having significantly different OVQ scores (Figs. 2 and 3). Chlorophyll and carotenoid concentrations were similar between food service and retail spinach.

Respiration rates from spinach grown in commercial fields or hydroponic culture were within the range (6 and 22 µl CO_2/g·h) reported by Cantwell et al. (1998) and Suslow and Cantwell (2009). However, respiration rates for field grown spinach within the first 5 days of evaluation (range 19.5-9.5 µl CO_2/g·h) were greater than those from hydroponic spinach (range 13-8.5 µl CO_2/g·h). No significant differences were observed between nitrogen treatments during 25 days of storage at 7.5°C. A 30% increase in nitrogen fertilization reduced sugar concentrations at harvest to values equal to or lower than 2 mg/g FW and this condition did not result in lower respiration rates. During

storage, greater carbon availability within the plant tissue has been correlated with higher respiration rates (Kader, 2002). This phenomenon was not observed. Kerstiens (1996) stated that epicuticular waxes, the cuticle, and epidermis are the outermost defensive barriers of leaves against plant abiotic and biotic stresses. No differences in cuticle thickness were observed between N treatments (data not shown); however, differences in total leaf and epidermis thickness, irrespective of leaf maturity, were associated with reduced shelf life (Tables 2 and 3; Fig. 2).

CONCLUSIONS

Spinach nutritional quality and postharvest shelf life is reduced significantly when cultivated at nitrogen concentrations greater than 125 ppm total N. Important phytonutrients including vitamin C and sugars were reduced in spinach from 200 ppm and 240 kg/ha total N at harvest. Respiration rates, green color, chlorophyll and carotenoid concentrations at harvest and during storage were poor indicators of the nutritional quality and shelf life of spinach. Differences in leaf and epidermis thickness between spinach cultivated with different nitrogen treatments were associated with low OVQ scores during storage. Although storage temperatures significantly affect quality retention in spinach, the initial quality at harvest is the main factor that influences postharvest outcomes. Spinach pre- and postharvest quality is best predicted by analysis of TOA, sugar concentrations and the relationship between ammonium and GS at harvest. There is still the need to develop a non-destructive and more practical tool that could predict quality at harvest and marketable shelf life.

ACKNOWLEDGEMENTS

Funding and in-kind contributions to this research provided by Fresh Express® Fresh Produce Safety Research Initiative and General Hydroponics. Gratitude for valuable input from Salinas Valley growers and conceptual contributions from Dr. Marita Cantwell. Authors recognize the technical efforts of Amy Gundersen and Cedric Bailey Ritezel.

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Tables

Table 1. Composition at harvest and during storage of spinach grown in hydroponic culture at two nitrogen concentrations. Data are averages of 8 replications per nitrogen treatment.

Nitrogen treatment (ppm total N)	Ascorbic acid (mg/100 g FW)			Total organic acids (mg/g DW)			Sugars[1] (mg/g FW)
	0 Day	10 Day	20 Day	0 Day	10 Day	20 Day	
125	54.6a	43.3c	32.3d	34.8d	35.1d	32.1e	2.3a
200	52.2b	44.7c	35.7d	42.1a	39.4b	37.1c	1.9a

[1] Sugar concentrations reported at harvest.
Different letters indicate significant differences between treatments within a specific phytonutrient, LSD.05.

Table 2. Anatomical characteristics at harvest of spinach leaves grown in hydroponic culture at two nitrogen concentrations. Data are averages of 8 replications per nitrogen treatment.

Nitrogen treatment (ppm total N)	Total leaf thickness[1] (μm)		Epidermis thickness[2] (μm)			
			Leaf 8		Leaf 16	
	Leaf 8	Leaf 16	Top	Bottom	Top	Bottom
125	796b	430b	29b	25a	22a	19a
200	857a	474a	33a	28a	21a	20a

[1,2] Letters indicate significant differences within a specific category evaluated, LSD.05.

Table 3. Anatomical characteristics of spinach leaves at harvest cultivated in field conditions with two nitrogen concentrations. Data are averages of 8 replications per nitrogen treatment.

Nitrogen treatment (kg/ha)	Total thickness[1] (μm)		Epidermis thickness[1] (μm)			
			Leaf 1-2		Leaf 3-4	
	Leaf 1-2	Leaf 3-4	Top	Bottom	Top	Bottom
190	639b	395b	26.5b	22b	20a	16.5a
270	787a	426a	29a	28a	21a	17a

[1,2] Letters indicate significant differences within a specific category evaluated, LSD.05.

Table 4. Relationship of OVQ* with vitamin C, chlorophyll and ammonium concentrations and decay of hydroponically grown spinach with two different N treatments during storage.

Parameters[1]	Regression analysis	r	Probability
OVQ- Decay	y=-3.513x+31.37	0.9327	0.007
OVQ-Vitamin C	y=13.45x-68.15	0.8128	0.01
OVQ-Chlorophyll	y=0.1499x-0.892	0.1809	0.29
OVQ-Ammonium	y=0.0123x-0.040	0.4815	0.33

* Overall visual quality.
[1] Regression analysis was done combining results for both N treatments per regression pair. A polynomial analysis was also used for OVQ, chlorophyll and ammonium. OVQ-Chlorophyll $y=-0.263x^2+4.44x-18.29$, r=0.27. OVQ-Ammonium $y=0.027x^2-0.429x+1.75$, r=0.82.

Figures

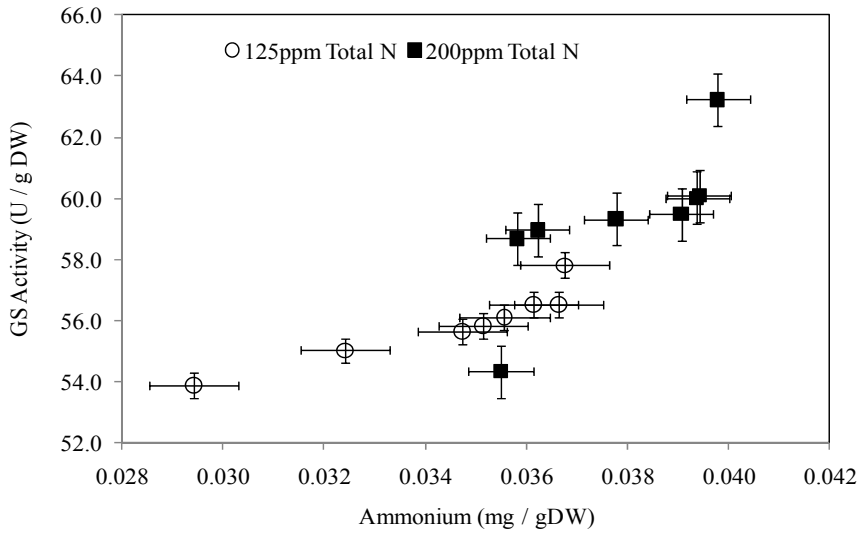

Fig. 1. Correlation between glutamine synthetase activity and ammonium concentrations at harvest of spinach grown in hydroponic culture. Data are averages of 3 repetitions per treatment.

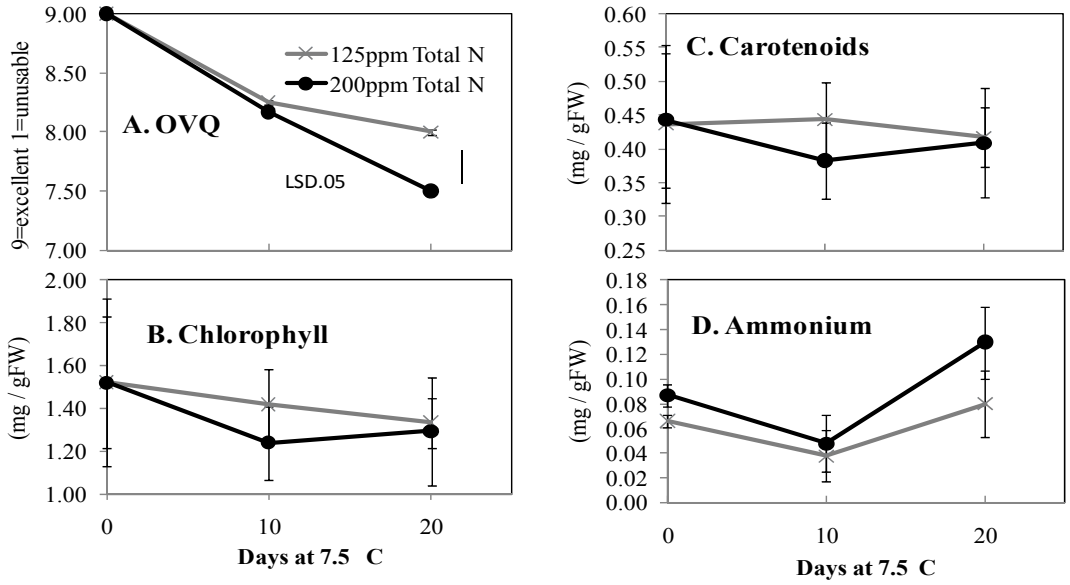

Fig. 2. Overall visual quality (OVQ), chlorophyll, carotenoid and ammonium concentrations of hydroponically grown spinach stored at 7.5°C for 21 days. Decay scores at 10 days were >1% and at 21 days were >2% in both treatments. Data are averages of 3 repetitions per treatment.

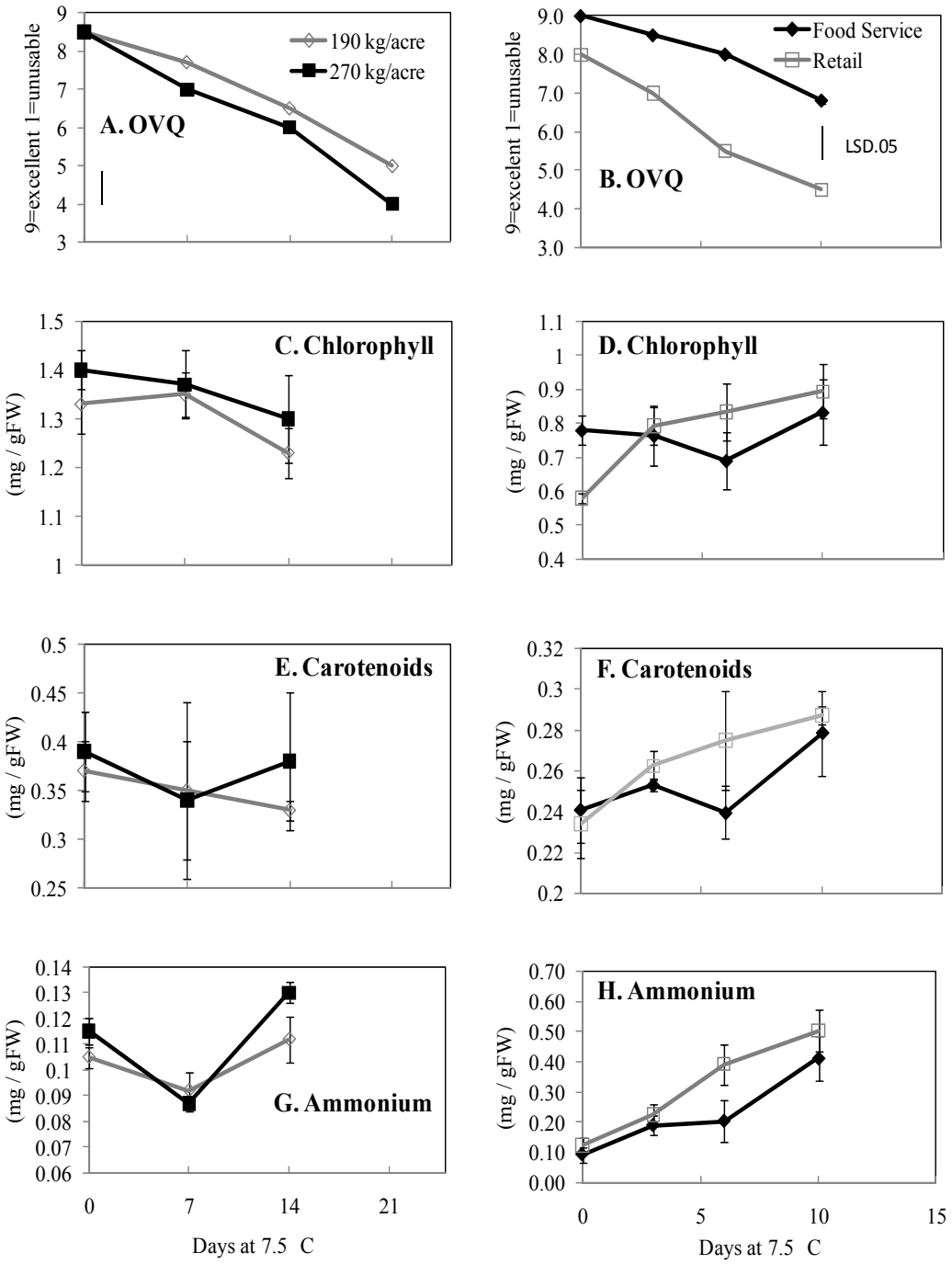

Fig. 3. Overall visual quality (OVQ), chlorophyll, carotenoids and ammonium concentrations of spinach from experimental plots (A,C,E,G) and commercial processing (B,D,F,H) stored at 7.5°C for 21 days. Data are averages of 3 repetitions per treatment. Decay scores after 10 days for retail and food service were greater than 10%. For field grown spinach after 15 days of storage, decay scores were greater than 5%. Vertical bars indicate LSD.05.